PGX TECHNOLOGY: NOVEL TAILOR-MADE AND TUNEABLE DELIVERY SYSTEMS FOR POORLY WATER-SOLUBLE BIOACTIVES

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ABSTRACT

The delivery of poorly water-soluble bioactives, including active pharmaceutical ingredients (API) and nutraceutical components is of great interest for existing drugs and new drug developments, cosmetic formulations, functional foods and nutraceuticals. This article presents a novel patented technology called PGX Technology, which utilizes pressurized gas expanded (PGX) liquids to dry, micronize, purify and functionalize water-soluble polymers. PGX Technology can generate open-porous nanostructured polymer carriers composed of one or several water-soluble polymers forming powders, granules, nano-fibrils, aerogels and exfoliated nano-composites with specific surface areas (SSA) ranging from tens to several hundred m^2/g. Such mesoporous water-soluble carrier systems can be impregnated with a bioactive by means of adsorptive precipitation, utilizing supercritical carbon dioxide, leading to the uniform deposition of nano-scale particles (<120 nm) throughout the porous matrix, forming a bioactive-polymer complex, for example coenzyme Q10 on β-glucan (CoQ10-iBG). A nano-dispersion of CoQ10 is formed when such CoQ10-iBG complex is dissolved in water, which is stable over 6 months at room temperature. The bioavailability of the CoQ10-iBG complex tested in rats compared favorably with a positive control (CoQ10 in triolein) and a commercial CoQ10-cyclodextrin complex.

Keywords: gas expanded liquid, biopolymer nano-fibers, nano-dispersion, solid dosage form

INTRODUCTION

Novel strategies for the formulation and processing of poorly water-soluble bioactives and drugs range from lipid-based colloidal drug delivery systems (DDS) to drug nanosuspensions and innovative solid dosage forms such as orodispersible films (ODFs) or electrospun fiber mats as drug carriers [Göke, 2018]. The generation of drug nanosuspensions can be achieved by either bottom-up or top-down methods. Top-down methods usually start from larger drug particles, which are then reduced in size by applying mechanical shear, force or cavitation treatment. Top-down methods employ milling techniques, such as wet milling, colloid milling, jet milling or high-pressure homogenization methods, such as micro-fluidization or a piston gap technique [Chin, 2014; Wais, 2016]. In a bottom-up method the drug is typically dissolved first in a suitable solvent and then precipitated by changing solubility, which can be achieved in several ways, such as the addition of an antisolvent. In such antisolvent precipitation processes the method of addition and mixing of the antisolvent to the solution becomes critical and can be achieved by means of rapid mixing devices, ranging from simple stirrers to rotating fixed beds, multi inlet vortex mixers and confined liquid impinging jets to reach highly turbulent and fast mixing thereby controlling supersaturation and nucleation [Chan, 2011]. Processing techniques using supercritical fluids (SCF) to produce drug nanoparticles/nanocrystals have also been developed, which take advantage of the highly tunable solvent properties of SCFs [Padrela, 2018]. The SCF in such process can be utilized as an antisolvent to cause precipitation out of a solution, a solvent for the solute or as an additive to facilitate atomization and drying [Padrela, 2018; Tabernero, 2012]. Moreover, SCF processes were developed for particle formation of polymers to be used as carrier delivery systems [Yeo, 2005]. For polymer processing, the SCF can be dissolved in a polymer melt and aid in melting point

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reduction and atomization, such as in the "Particle formation from gas-saturated solution" (PGSS) process, leading to highly porous polymer particles and agglomerates [Yeo, 2005].

Pressurized Gas eXpanded (PGX) liquid Technology has been developed at the University of Alberta [Seifried, 2010], is patented in the US, Europe and Canada [Temelli & Seifried, 2016 and 2018] and has been scaled-up to demo-scale at Ceapro Inc. [Seifried, 2019]. This platform technology can purify and dry aqueous solutions or slurries of polymers into open-porous nano-structured particles and fibrils utilizing a PGX fluid composed of supercritical carbon dioxide ($SCCO_2$) and anhydrous ethanol at mild operating conditions of 40°C and 100 bar as drying fluid. With the PGX Technology, single and multiple polymers can be processed by simply blending polymer solutions or suspensions to generate exfoliated nano-composites. As well, the water-soluble polymers can be further modified by cross-linking during the PGX process, allowing generation of tailor-made and tunable delivery systems.

The first objective of this work is to present various polymers processed by PGX Technology into open-porous particles, fibrils and nano-composites with large specific surface area (SSA). The next objective is to demonstrate that such open-porous PGX biopolymer carriers can be loaded with a bioactive forming novel bioactive impregnated polymer complexes termed "**i**mpregnated **P**olymer comple**X**" (iPX) by means of adsorptive precipitation utilizing $SCCO_2$ [Gurikov, 2018]. Specifically, PGX processed oat β-glucan was loaded with the bioactive Coenzyme Q10 (CoQ10), a poorly bioavailable lipophilic substance with antioxidant activity. The new complex termed CoQ10-iBG is compared to a commercial CoQ10-cyclodextrin complex in terms of water dispersibility and physical nanosuspension stability. Finally, a pilot study demonstrating the intestinal bioavailability of CoQ10 was performed to determine the direct in-vivo intestinal bioavailability of CoQ10 from the CoQ10-iBG complex compared to a standard food grade and commercial crystalline CoQ10 formulations using an intestinal-lymph cannulated rodent model.

RESEARCH CONCEPT

Generation of polymer carrier: PGX Technology involves the spraying of an aqueous solution or slurry of polymer into a pre-pressurized collection chamber, together with a PGX fluid, composed of $SCCO_2$ and a water-soluble co-solvent/antisolvent (i.e. ethanol, acetone or isopropanol) by means of a coaxial nozzle. At the processing conditions, the PGX fluid and aqueous phase become completely miscible thereby eliminating interfacial tension and capillary forces leading to rapid precipitation of the polymer and quick removal of the water. Thus, open-porous and unique nanoscale morphologies can be generated.

Loading of bioactive onto polymer carrier: The porous polymer carrier generated by PGX is then subjected to an adsorptive precipitation process utilizing $SCCO_2$ to load a bioactive or drug onto the matrix [Gurikov, 2018]. In this process, the bioactive is essentially solubilized at elevated pressure and suitable temperature in $SCCO_2$ and passed through a bed of porous polymer carrier previously prepared using the PGX Technology. During this step, the SCCO2 with the dissolved bioactive or drug can penetrate the entire carrier matrix deep into the porous structure, where it can interact with the polymer matrix, leading to adsorption of the drug on the polymer. Furthermore, by modulating the pressure during the adsorptive precipitation process a sudden change of solute solubility in the $SCCO_2$ solution can be rapidly induced, leading to precipitation of nano-scale deposits of the bioactive throughout the matrix [Gurikov, 2018]. For this study, an open-porous polymer carrier was generated by PGX Technology from oat β-glucan (BG) and then loaded with CoQ10 by means of adsorptive precipitation as described and characterized elsewhere [Liu, 2018; Couto, 2018].

Helium Ion Microscopy: For imaging the nanoscale features of the various PGX-processed polymers and impregnated CoQ10-iBG complex a Helium ion microscopy (HiM) analysis was performed on a Zeiss Orion NanoFab Helium Ion Microscope (Ostalbkreis, BW, Germany). Secondary Electron (SE) images were collected at 30 kV accelerating voltage and 1.5 pA beam current. An electron flood gun was utilized to neutralize positive charges accumulated on the sample surfaces, which enables direct imaging of insulating materials.

Stability of aqueous CoQ10 dispersions: Aqueous dispersions of the hydrophobic bioactive CoQ10 were prepared by dissolving either a commercial CoQ10-γ-cyclodextrin (CoQ10-CD) complex (CAVAMAX®, containing 20% w/w of CoQ10) or the CoQ10-iBG complex loaded with 8% w/w of CoQ10 in about 250mL of reverse osmosis (RO) water aiming at a CoQ10 concentration of 0.4 mg/mL by slowly dispersing the

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powder into pre-heated RO water at 40°C in beakers and mixing with a magnetic stirrer. The beakers were then covered with parafilm and left to cool to room temperature on the lab bench. The dispersions were left at room temperature under a dark box to prevent light exposure and observed up to 6 months.

Bioavailability Study: The study design and experimental protocol for the intestinal bioavailability of CoQ10 from different formulations is shown in Figure 1.

CoQ10 formulations: The bioavailability of CoQ10 was tested using four formulations: i) 3% w/w CoQ10-iBG (n=3) pilot test, ii) 8% w/w CoQ10-iBG (n=9), iii) 20% w/w CoQ10-CD product (n=6) and iv) Food grade CoQ10 in triolein (n=6). Each formulation (i-iii) was prepared by dispersing the sample in deionized distilled water heating and mixing in a water bath at 45°C for 35 min and cooling to room temperature (~23°C). Since the food grade crystalline CoQ10 is insoluble in water, it was prepared in triolein (1mL), which represents a positive control for absorption of this lipophilic nutrient. Each formulation of CoQ10 was administered intra-gastrically at a dose of ≈10 mg/kg body weight based on CoQ10 bioavailability studies in animal and rodent models [Hatanaka, 2008; Barakat, 2013].

Intestinal bioavailability: The intestinal bioavailability of CoQ10 from the CoQ10-iBG complex and standard formulations was evaluated using surgical cannulation of the stomach and intestinal mesenteric lymphatic vessel in a rodent model, which is a direct measure of the absorption of lipophilic nutrients and compounds, as previously described [Wang, 2012]. Intestinal lymph was collected into tubes containing EDTA and stored at -80°C for HPLC analyses.

HPLC Analysis of Ubiquinones: HPLC methods were established by modifying previously described methods for CoQ9 and CoQ10 [Schulz, 2006; Tarry-Adkins, 2016; Boitier, 1998; Mosca, 2002]. The phylloquinone Vitamin K was used as an internal standard and the quantitation of CoQ9 and CoQ10 was performed using commercial standards to identify and detect these ubiquinones in intestinal lymph samples. In brief, total lymph ubiquinone (CoQ9 and CoQ10) was oxidized with 1,4-benzoquinone, and extracted with 1-propanol and then analyzed by reverse-phase HPLC (Agilent Poroshell 120, 4.6x100 mm) with UV detection at 275 nm. Peak areas were analyzed, and CoQ9 and CoQ10 were quantified using vitamin K as an internal standard (1mg/200 mL sample). Validation of the HPLC protocol was based on linear calibration curves of each standard and 99-100% recovery of the standards; vitamin K, CoQ9 and CoQ10 (Thermo Fisher Scientific, DC chemicals, Sigma-Aldrich).

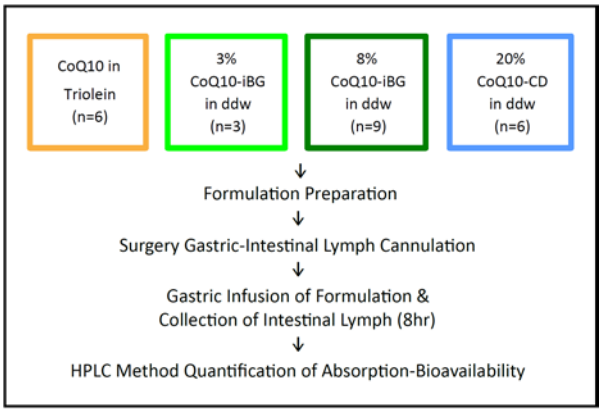

Figure 1: Study design and experimental protocol for determining the bioavailability of CoQ10 formulations.

Bioavailability and Pharmacokinetics: % Total absorption of CoQ10, CoQ10 area under the curve (CoQ10-AUC), maximum concentration (cMax) were determined as previously described [Barakat, 2013; Wang, 2012]. % Absorption was adjusted per volume of lymph and per hour of collection to reflect pharmacokinetics of lymph absorption-bioavailability [Wang, 2012; Barakat, 2013].

RESULTS

To demonstrate the capabilities of PGX Technology, several biopolymers were processed into open-porous structures, which can be impregnated with drugs or bioactives and utilized as bioactive delivery systems. Some examples are illustrated in Figure 2, showing PGX processed corn starch, pectin, sodium alginate, and an alginate-pectin exfoliated composite.

The PGX processed oat β-glucan is displayed in Figure 3(A) illustrating the open-porous structure. The BG carrier was impregnated to generate the CoQ10-iBG complex with spherical nanocrystals of CoQ10 with an average size of about 92 nm (Figure 3(B)).

The stability of CoQ10 dispersions in water at room temperature is illustrated in Figure 4 after about 18 hours (left image) and after 6 months (right image). The CoQ10-iBG complex dispersion remained stable for more than 6 months at room temperature.

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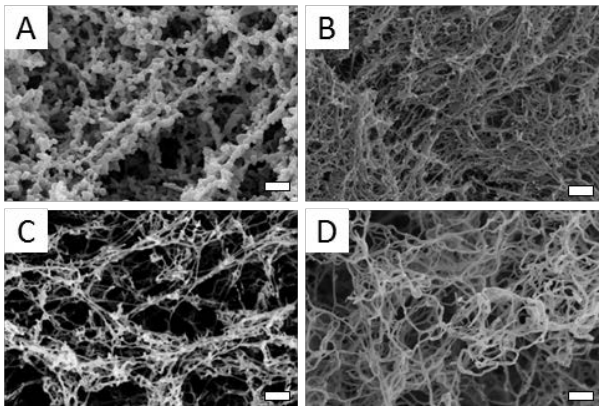

Figure 2: HiM images of (A) PGX processed corn starch, (B) pectin, (C) sodium alginate, and (D) alginate-pectin nano-composite. Scale bar length: 200 nm.

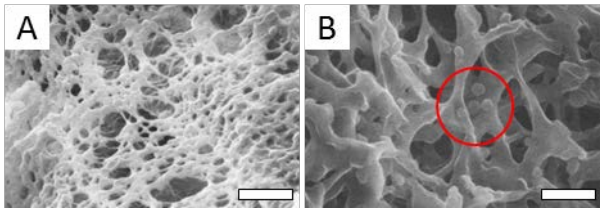

Figure 3: HiM images of (A) open-porous polymer carrier generated by PGX Technology from oat β-glucan, and (B) CoQ10-iBG complex with nanocrystals of CoQ10 inside red circle (source: [Liu 2018]). Scale bar length: 500 nm.

Figure 4: Stability of CoQ10 dispersions in water, majority of commercial CoQ10-CD (left beaker) precipitated in 18.35 hrs (left image), whereas the CoQ10-iBG complex (right beaker) was stable for more than 6 months (right image).

Bioavailability of CoQ10 formulations: The results of the bioavailability study are shown in Table 1.

Table 1: CoQ10 bioavailability results for the different formulations used in the rodent model.

	CoQ10-triolein	*3% CoQ10-iBG*	*8% CoQ10-iBG*	*20% CoQ10-CD*
% Total Abs.	2.43 ±1.01	1.71 ±0.93	4.06 ±2.12	3.27 ±2.06
c_{Max} *(mg/ml)*	53.10 ±6.23	27.54 ±7.21	63.82 ±31.04	56.97 ±24.38
CoQ10-AUC (mg/ml)	67.23 ±35.42	55.63 ±33.53	136.90 ±73.32	133.3 ±65.82

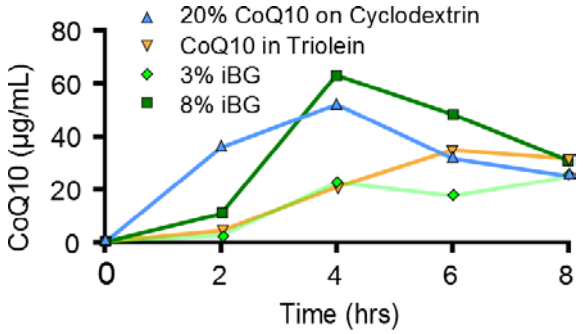

Figure 5: Absorption kinetics of CoQ10 formulations.

DISCUSSION

The CoQ10 area under curve (CoQ10-AUC) was >50% higher for both the 8% CoQ10-iBG and 20% CoQ10-CD formulations compared to the 3% CoQ10-iBG and Food CoQ10 in triolein formulations (Table 1). The data show that both BG and cyclodextrin formulations increase the absorption of the lipophilic nutrient CoQ10; however, the kinetic profile was different with the 8% CoQ10-iBG formulation having a delayed maximal absorption compared to the 20% CoQ10-CD (Figure 5). This may reflect overall faster digestion, including gastric emptying of the 20% CoQ10-CD compared to the 8% CoQ10-iBG formulation most likely due to the higher viscosity of the β-glucan. Due to the fine particles of CoQ10 deposited on the BG carrier the bioavailability could be enhanced as well, which would agree with a previous study where CoQ10 nanocrystals with 80 nm diameter had a 7.3-fold increased bioavailability compared to a coarse suspension of the bulk drug [Sun, 2012].

CONCLUSIONS

This work demonstrates the capabilities of PGX Technology to generate tailor-made and tunable bioactive carrier/delivery systems from water-soluble biopolymers. Such biopolymers can be impregnated with lipophilic bioactives utilizing $SCCO_2$ assisted adsorptive precipitation forming novel iPX formulations. Resulting iPX are water dispersible and form stable nanodispersions, which, in the case of CoQ10, were shown to be more bioavailable. Use of other polymers, polymer combinations, and cross-linked polymers can lead to improved delivery and release profiles of drugs/bioactives, which is our ongoing investigation.

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